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A BINARY MUTABILITY SYSTEM IN ESCHERICHIA COLI

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Although genetic instability is often largely a function of the gene which itself mutates, there is abundant evidence that the residual genome may exert an effect. This effect may be strong and highly localized, as if at a single locus.[1] Such binary mutability systems in maize have been investigated extensively and have led to important concepts of gene mutation and cellular differentiation.[2] Among microorganisms, differences in spontaneous mutation rates have been analyzed genetically in only a few instances,[3] and in no case has a binary system been revealed. Treffers *et al.*[4] discovered that one substrain of *Escherichia coli*, strain K-12, exhibits a mutation rate from streptomycin sensitivity to streptomycin resistance that is about 100-fold greater than that observed in other K-12 substrains. The present investigation reveals that the basis of mutability in this strain is binary, involving genetic determinants which are not closely linked.

MATERIALS AND METHODS

The mutable strain, CS19, employed in these experiments was derived from 58-278,[4, 5] but no longer requires biotin. Strain CS19 carries the following pertinent markers: F^+ Pa^- T^+ L^+ Th^+ Lac_1^+ S^s V_1^s Mut^+. Additional K-12 substrains employed are W677 (F^- Pa^+ T^- L^- Th^- Lac_1^- S^s V_1^r Mut^-), W1177 (an S^r derivative of W677), and CS11 (an F^+ derivative of W1177). The designations F^+ and F^- refer to mating type;[6] Pa^-, T^-, L^-, and Th^-, to phenylalanine, threonine, leucine, and thiamine growth requirements, respectively; Lac^-, to an inability to ferment lactose; S^r and V_1^r to resistance to streptomycin and coliphage T1 (and T5). The determinant of mutability is symbolized as Mut^+. Like other nonmutable strains, the rate with which W677 undergoes mutation from S^s to S^r is about 4×10^{-10} per bacterial division, whereas the CS19 mutation rate is about 4×10^{-8}.

The B/r strains employed have been described elsewhere[7] and may be characterized here by the location of their auxotrophic mutations with relation to two important and more or less distinct segments of the *E. coli* genome.[8] IMN27 requires arginine and methionine because of two mutations in the *M—Th* region; IMN64 requires tryptophan, tyrosine, phenylalanine, and *p*-aminobenzoic acid, owing to a single mutation in the *S—Xyl* region. Both are F^- S^s Mut^-.

The experimental procedures employed follow descriptions given elsewhere.[9] For crosses, 16–20-hour Difco Penassay broth cultures were washed twice in saline, and 0.1-ml. samples were mixed on minimal (or supplemented minimal) agar medium. Recombinants were examined after single-colony isolation on complete medium. Streptomycin, threonine, leucine, and thiamine were made up to concentrations of 200, 20, 10, and 0.01 mg/l, respectively, where indicated.

To score recombinants for mutability, about 100 cells were inoculated into 10 ml. of Penassay broth and incubated for 24 hours at 37° C. without aeration. Then 0.1-ml. samples were plated onto nutrient agar containing streptomycin. If 10 or more resistant colonies appeared, the recombinant was scored as mutable. If less than 10 colonies appeared, two new broth cultures were initiated from small inocula and similarly assayed; if each of these two samples contained less than 10 resistant cells, the recombinant was scored as nonmutable. In no case was further testing necessary. This is sufficient to distinguish parental types with a low probability of error, since it was found empirically that among samples of 50 W677 cultures similarly grown, 45 contained no resistant cells and none contained more than 4, whereas, among samples of 50 CS19 cultures similarly grown, none contained less than 25 resistant cells.

RESULTS

The Site of Instability Determination.—CS19 was crossed with W677 on unsupplemented medium and medium singly supplemented with threonine, leucine, and thiamine. Recombinants were scored for *Lac*, V_1, *Mut*, and unselected nutritional markers. Within the limitations of the method of testing, the segregation of mutability was unambiguous. In the unsupplemented cross (Table I), the high frequency of mutable recombinants indicates a close linkage of *Mut* to *T*, *L*, or *Th*. In the thiamine-supplemented cross, the equally high incidence of mutability among both the Th^+ and Th^- recombinants indicates that the close linkage is not

to *Th*. A comparison of the L^- and T^- recombinants from the third and fourth crosses reveals that, whereas 5 out of 8 T^- recombinants are mutable, only 2 out of 24 L^- recombinants are mutable. Thus *Mut* appears to be more closely linked to *L* than to *T*. The sequence Lac_1—V_1—*L*—*T* is extensively documented[10] and is consistent with the present data. A decision as to whether *Mut* lies to the left or to the right of *L* is not easily made on the basis of the limited data available. However, the fact that, of the 9 nonmutable recombinants issuing from the threonine-supplemented cross, all involve a crossover between V_1 and *L*, whereas only 3 involve a crossover between *L* and *T*, suggests that *Mut* lies to the left of *L*. If so, the frequencies of single crossovers in the three regions, $Lac_1 \overset{1}{—} V_1 \overset{2}{—} Mut \overset{3}{—} L$, provide a crude estimate of relative map distances. Considering only prototrophic recombinants and excluding those obtained in the presence of leucine, these frequencies are 73, 22, and 6, respectively. Prototrophs obtained on leucine are excluded because of the suggestion that some of them may be inhibited by this amino acid.[10]

TABLE 1

SEGREGATION OF *Mut* AND ASSOCIATED FACTORS IN CROSSES BETWEEN CS19 (F^+ Pa^- T^+ L^+ Th^+ Lac_1^+ V_1^s S^s Mut^+) AND W677 (F^- Pa^+ T^- L^- Th^- Lac_1^- V_1^r S^s Mut^-)

Unselected Markers				Supplement					
			None	Thiamine		Leucine		Threonine	
Lac	V_1	*Mut*		Th^+	Th^-	L^+	L^-	T^+	T^-
+	*s*	+	15	5	9	25	2	49	1
−	*s*	+	18	4	16	21	0	51	3
−	*r*	+	6	2	6	6	0	14	1
+	*r*	+	0	0	0	2	0	1	0
+	*s*	−	0	0	0	0	1	0	0
−	*s*	−	0	0	0	0	1	0	0
−	*r*	−	1	0	1	1	20	5	2
+	*r*	−	0	0	0	0	0	1	1
Total			40	43		77		129	

The Site of Mutation.—Two independent S^r derivatives of CS19 were crossed with W677. Owing to the much higher incidence (at least 100×) of S^r mutants in Mut^+ cultures than in Mut^- cultures, it is likely that the S^r mutations were associated with Mut^+. The frequency of mutable types among the S^s recombinants (Table 2) was comparable to that observed in the cross of CS19 S^s with W677, indicating that mutation from S^s to S^r does not inactivate Mut^+. Further, very few of the recombinants were S^r, indicating not only the binary nature of the mutability system but also that the components are not closely linked.

The absence of S^r recombinants in a cross of one CS19 S^r with W1177 (S^r) suggested that the two mutations were isolocal or closely linked. The question whether most of the S^r mutations in Mut^+ populations also occur at this site was approached by a somewhat more sensitive test. The aromatic deficiency of the B/r strain, IMN64, is closely linked to the *S* locus (of W1177), whereas the deficiencies of IMN27 are not. In K-12 (F^+) × B/r (F^-) crosses, only those K-12 unselected markers which are closely linked to the deficiency (selective marker) of the B/r parent occur frequently among recombinants. Thus the frequency of S^r recombinants from the cross of CS11 (W1177 F^+) and IMN64 is higher than from the cross of CS11 and IMN27 (Table 2). Six independent S^r mutants of CS19

were crossed with IMN64. The high frequency of S^r recombinants in all these crosses is consistent with the conclusion that most Mut^+-associated mutations to

TABLE 2

SEGREGATION OF *Mut* AND *S* IN SELECTED CROSSES

F^+ PARENT	F^- PARENT	PROTOTROPHIC RECOMBINANTS EXAMINED	PER CENT S^r	PER CENT S^s Mut^+	PER CENT S^s Mut^-
CS19 S^r #1	W677	40	13	100	0
S^r #2	W677	64	22	96	4
CS11	IMN64	72	56	...	...
	IMN27	48	17	...	...
CS19	IMN64	48	...	6	94
	IMN27	48	...	4	96
CS19 S^r #2	IMN64	50	60	...	...
S^r #3	IMN64	50	70	...	...
S^r #4	IMN64	50	60	...	...
S^r #5	IMN64	50	62	...	...
S^r #6	IMN64	50	62	...	...
S^r #7	IMN64	50	58	...	...
CS19 S^r #2	IMN27	50	18	...	...

streptomycin resistance occur at or near the same site. Since the deficiencies of IMN64 and IMN27 are not closely linked to *L*, the low frequency of Mut^+ recombinants in crosses of these strains with CS19 is expected.

DISCUSSION

Examination of mutable recombinants from crosses involving CS19 immediately revealed that mutants other than S^r were abnormally frequent. This is also true of CS19 populations.[4] The range of action of Mut^+ is not the immediate concern of this report, but it is appropriate to remark that the abnormally frequent mutants include Th^+ in Th^- Mut^+ recombinant populations and T^+ in T^- Mut^+ recombinant populations. If these represent reversions, three widely separated loci are Mut^+-influenced. The remoteness of the two components of the *S* mutability system lends credence to the view that instability is manifested throughout the bacterial genome in this mutable strain and possibly in others.[11] This approximates the situation in *Drosophila* populations containing *mu-F*[12] and is not inconsistent with the situation expected of maize populations containing *Ac* and *Ds*.[2]

That Mut^+ acts by inducing mutations has been assumed in this presentation. Evidence has been presented[4] that differences in relative growth rates cannot explain the high frequency with which S^r mutants appear in Mut^+ strains. The objection that mutations might be equally frequent in Mut^- strains, but more often lethal, is applicable, of course, to many situations where differences in mutation rates are inferred.

SUMMARY

The high rate of spontaneous mutation to streptomycin resistance (S^r) exhibited by the K-12 substrain CS19 has been shown to involve a binary mutability system. Mutations occur at or near the same *S* locus, but their high frequency depends upon a remotely linked factor, Mut^+. The segregation of Mut^+/Mut^- is well defined and is consistent with a close linkage to the *L* (leucine) locus.

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